AD_____

AWARD NUMBER: W81XWH-04-1-0802

TITLE: Bioenergetic Approaches and Inflammation of MPTP Toxicity

PRINCIPAL INVESTIGATOR: M. Flint Beal, M.D.

CONTRACTING ORGANIZATION: Cornell University
New York, NY 10065

REPORT DATE: September 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



**1. REPORT DATE**
1 September 2009

**2. REPORT TYPE**
Annual

**3. DATES COVERED**
1 Sep 2008 – 31 Aug 2009

**4. TITLE AND SUBTITLE**

Bioenergetic Approaches and Inflammation of MPTP Toxicity

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-04-1-0802

**5c. PROGRAM ELEMENT NUMBER**

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**6. AUTHOR(S)**

M. Flint Beal, M.D.

E-Mail: fbeal@med.cornell.edu

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Cornell University
New York, NY 10065

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

This project was to develop novel therapies for Parkinson's Disease (PD), to test the effects of human dopaminergic stem cells, and to utilize metabolomic profiling to develop biomarkers for PD. We also developed a new transgenic mouse model of PD by knocking out the gene PINK1. Utilizing microdialysis we showed that PINK1 deficient mice have impaired dopamine release. We demonstrated that rolipram, mitochondrial targeted antioxidant peptides, celastrol and promethazine were neuroprotective against MPTP toxicity. We showed that novel forms of Coenzyme Q, exert neuroprotective effects. We demonstrated that MMP3 knockout mice were protected against MPTP toxicity. Utilizing microdialysis, we showed that PINK1 deficient mice have impaired dopamine release. We showed that human dopaminergic stem cells reverse deficits in the 6-hydroxy-dopamine model of PD. We carried out studies using metabolomic profiling to develop biomarkers for PD. We utilized metabolomic profiling with high performance liquid chromatography coupled to electrochemical coulometric array detection. In these studies, we were able to completely separate unmedicated PD subjects from controls. We subsequently were able to clearly separate PD patients with the LRRK2 mutations from idiopathic PD and controls. Similarly, we could separate gene positive patients from both controls and LRRK2 mutation negative patients. Lastly, we studied a number of agents which produced significant neuroprotective effects against MPTP toxicity and which therefore might be useful in human clinical trials to treat PD.

**15. SUBJECT TERMS**
No Subject Terms were provided.

**16. SECURITY CLASSIFICATION OF:**

| a. REPORT | b. ABSTRACT | c. THIS PAGE |
|---|---|---|
| U | U | U |

**17. LIMITATION OF ABSTRACT**
UU

**18. NUMBER OF PAGES**
13

**19a. NAME OF RESPONSIBLE PERSON**
USAMRMC

**19b. TELEPHONE NUMBER** *(include area code)*

# Table of Contents

## 4. INTRODUCTION

This is a project, which has been ongoing since September of 2004. The grant initially was designed to study neuroprotective agents in an MPTP model of Parkinson's Disease (PD), as well as the pathophysiology of mitochondrial dysfunction in PD. We modified the proposal to characterize a new animal model of PD made by knocking out PINK1, a nuclear encoded kinase localized to mitochondria, and to study the effects of human dopaminergic stem cells in a 6-hydroxydopamine (6-OHDA) model of PD. More recently, we revised our efforts to focus on the development of metabolomic profiling to identify biomarkers for PD. These studies are ongoing and we have been able to make considerable progress in this area over the last year.

Outline of Research Goals:

Task 1: To determine the ability of pharmacologic agents to prevent MPTP neurotoxicity.

Task 2: To develop a new transgenic mouse model of PD by knocking out PINK1, a protein in which mutations can cause autosomal recessive PD.

Task 3: To utilize metabolomic profiling to develop biomarkers for PD.

Task 4: To determine the efficacy of human dopaminergic stem cells in the 6-hydroxydopamine model of PD.

## 5. BODY

**Task 1:** To determine the ability of pharmacologic agents to prevent MPTP neurotoxicity. These studies have been largely completed.

We did studies using the phosphodiesterase inhibitor rolipram. This leads to an increase in cAMP and phosphoCREB, which is neuroprotective. We saw protective effects with either 1.2 or 2.5 mg/kg. We showed significant protection against loss of dopamine and depletion of tyrosine hydroxylase neurons. These results were published in *Experimental Neurology.*

We also examined neuroprotective effects of mitochondrial-targeted antioxidants (SSO2 and SS31), which inhibit the mitochondrial permeability transition (MPT). We made substantial progress in these studies. We found that both of the compounds which are highly concentrated to mitochondria have ROS scavenging properties and were able to dose-dependently inhibit lipid peroxidation as measured by chemoluminescence. We also found that these compounds protect against MPP+ toxicity *in vitro.* We carried out studies against MPTP toxicity in mice. We found that both compounds were able to significantly protect against MPTP induced loss of dopamine, as well as tyrosine hydroxylase neurons. Dopamine metabolites DOPAC and HVA showed similar effects. This work has been recently published in a manuscript in *Antioxid Redox Signal.*

We examined neuroprotective effects of celastrol and promethazine on MPTP. These studies were completed and they showed marked neuroprotective effects. We also worked on the mechanism of these agents. Celastrol upregulates HSP70 and promethazine blocks the MPT. These results were published in the *Journal of Neurochemistry* and in *Neurobiology of Disease* respectively. We also examined novel forms of Coenzyme Q10 (CoQ10) in the MPTP model.

We carried out a large number of studies, which showed that there were indeed significant protective effects. In particular, we were able to show that CoQ administered in a chronic model of MPTP toxicity not only protected against loss of tyrosine hydroxylase neurons, but it also protected against the development of alpha-synuclein aggregates. This was a model in which MPTP was administered over one month by Alzet pump. These results we believe are particularly relevant to PD itself. These results are now published in the *Journal of Neurochemistry.*

We also examined the role of caspase 3 activation and activation of microglia and MPTP toxicity. We studied the effects of matrix matalloprotease 3 (MMP3) and its role as a novel signaling proteinase from apoptotic neuronal cell death, which results in activated microglia. We found that MMP3 knockout mice were protected against MPTP toxicity. We also found that MMP3 was important in activating

NADPH oxidase to generate superoxide and that this plays a direct role in dopamine cell death. These results were published in the *FASEB Journal.*

**Task 2**: To develop a new transgenic mouse model of PD by knocking out PINK1, a protein in which mutations can cause autosomal recessive PD.

We generated the PINK1 knockout mice. Correctly targeted ES cells were used to inject and generate PINK1 knockout mouse founders. We are now continuing to expand the colony. We found mild defects in motor behavior and we also found impaired dopamine release using microdialysis. We also observed a number of different phosphorylated proteins on two-dimensional gels. Of particular interest, the protein OMI was phosphorylated by PINK1 and this has been confirmed by other authors. Further characterization of these mice is continuing.

**Task 3**: To utilize metabolomic profiling to develop biomarkers for PD.

The primary aim of our ongoing studies and existing protocol is to determine whether neurochemical markers in blood or spinal fluid can be used to make early diagnoses or to follow the progression of PD. The underlying hypothesis of the existing protocol is that disordered energy metabolism may contribute to the pathogenesis of neurodegenerative diseases, and in particular, PD. We, therefore, studied both cell lines as well as body fluids from patients with PD as well as normal controls. We carried out initial studies in 25 controls and 66 PD subjects. We utilized metabolomic profiling with high performance liquid chromatography, coupled with electrochemical coulometric array detection. In these studies, we were able to completely separate unmedicated PD subjects from controls. This work was published in *Brain.* We carried out further studies of patients and gene carriers with LRRK2 mutations, which is responsible for autosomal dominant inherited PD. This enabled us to identify the variables, which were responsible for the separations in the LRRK2 PD subjects and family members who are carriers of this genetic defect, as well as patients who have manifest PD. We can clearly separate PD patients with LRRK2 mutations from idiopathic PD and controls. Similarly, we can separate gene positive patients from both controls and LRRK2 mutation negative patients. We are now working on structural elucidation of the remaining analytes separating PD patients from controls using mass spectroscopy. We are also using an integrated parallel LCECA/LCMS device with post-column splitting between electrochemical and mass spectrometric detectors. We intend to expand these studies into a much larger cohort of patients to determine the sensitivity and specificity of these analyses as biomarkers for PD diagnosis and assessment of therapies.

**Task 4**: To determine the efficacy of human dopaminergic stem cells in the 6-hydroxydopamine model of PD.

These studies were completed. We showed that the stem cells survived readily. The acquisition of highly enriched dopaminergic populations is an important prerequisite to human embryonic stem cell (HES)-derived dopaminergic neurons

for cell-based therapy. We utilized a new strategy improving the efficiency of dopaminergic neurogenesis from human ES cells. This involved co-culture with telomerase immortalized human mesencenphalic astrocytes during induction of a dopaminergic phenotype using sonic hedgehog and FGF8. Utilizing these enriched dopaminergic neurons, we were able to achieve a substantial and long-lasting restitution of motor function in 6-OHDA-lesioned adult rats. We also showed effective generation of TH positive neurons in all six animals studied. We examined BDRU incorporation, which showed that there was a small percentage of neurons, which showed BDRU incorporation consistent with evidence of mitosis. These extremely promising results are important in setting the stage for further work on human transplantation. These studies were published in *Nature Medicine.*

## 6. KEY RESEARCH ACCOMPLISHMENTS

A. We demonstrated that several novel pharmacologic agents exert neuroprotective effects in the MPTP model of PD.

B. We have developed a knockout model of PINK1. We are continuing to study these mice to enable a full characterization.

C. We showed that HES-derived dopaminergic neurons exert beneficial effects in the 6-hydroxy-dopamine models of PD.

D. We have continued metabolomic profiling and now have shown that we can separate unmedicated PD patients from controls, as well as medicated PD patients from controls. This work has now been published. We also showed that a number of specific metabolites were altered including 8-hydroxy-2-deoxyguanosine and reduced uric acid. We have now shown that we can separate idiopathic PD patients from PD patients wit the LRRK2 mutation. In addition, we can separate LRRK2 gene-positive carriers from both controls and LRRK2 mutation-negative patients.

## 7. REPORTABLE OUTCOMES

Cleren C, Calingasan NY, Chen J, Beal MF. Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity. J Neurochem 2005;94:995-1004

Cleren C, Starkov AA, Calingasan NY, Lorenzo BJ, Chen J, Beal MF. Promethazine protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. Neurobiol Dis 2005;20:701-708

Abeliovich A, Beal MF. Parkinsonism genes: culprits and clues. J Neurochem 2006;99:1062-1072

Lin MT and Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006;443:787-795

Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 2006;12:1259-1268

Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang L, Beal MF, Vogel H, Lu B. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink 1 is reduced by Parkin. Proc Natl Acad Sci USA 2006;103:10793-10798

Kim YS, Choi DH, Block ML, Lorenzl S, Yang L, Kim YJ, Sugama S, Cho BP, Hwang O, Browne SE, Kim SY, Hong JS, Beal MF, Joh TH. A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration microglial activation. FASEB J. 2007;21:179-187

Thomas B and Beal MF. Parkinson's disease. Hum Mol Genet 2007;Oct 15;16 Spec No. 2: R183-194. Review

Bogdanov M, Matson WR, Wang L, Matson T, Saunders-Pullman R, Bressman SS, Beal MF. Metabolomic profiling to develop blood biomarkers for Parkinson's disease. Brain 2008;131:389-396

Chaturvedi RK and Beal MF. PPAR: a therapeutic target in Parkinson's Disease. J Neurochem 2008;106:506-518

Cleren C, Yang L, Lorenzo B, Calingasan NY, Schomer A, Sireci A, Wille EJ, Beal MF. Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. J Neurochem 2008; 104:1613-1621

Yang L, Calingasan NY, Lorenzo BJ, Beal MF. Attenuation of MPTP neurotoxicity by rolipram, a specific inhibitor of phosphodiesterase IV. Exp Neurol 2008;211:311-314

Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H, Lu B. Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. Proc Natl Acad Sci USA 2008;105:7070-7075

Yang L, Zhao K, Calingasan NY, Luo G, Szeto HH, Beal F. Mitochondria targeted peptides protect against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine neurotoxicity. Antioxid Redox Signal 2009, Feb 9 (Epub ahead of print)

## 8. CONCLUSION

We accomplished our original research goals. We have characterized a number of agents, which show protection against MPTP toxicity. We have developed a new transgenic mouse model of PD by knocking out PINK1. We are continuing metabolomic profiling studies of PD patients and we have found that we can identify unique biomarkers in patients with LRRK2 mutations. We completed studies of transplantation of human ES cell derived dopaminergic neurons into a 6-hydroxy-dopamine model of PD and have shown successful restitution of behavioral abnormalities.

## 9. REFERENCES

None.

## 10. APPENDICES

Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 2006;12:1259-1268

Kim YS, Choi DH, Block ML, Lorenzl S, Yang L, Kim YJ, Sugama S, Cho BP, Hwang O, Browne SE, Kim SY, Hong JS, Beal MF, Joh TH. A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration microglial activation. FASEB J. 2007;21:179-187

Thomas B and Beal MF. Parkinson's disease. Hum Mol Genet 2007;Oct 15;16 Spec No. 2: R183-194. Review

Bogdanov M, Matson WR, Wang L, Matson T, Saunders-Pullman R, Bressman SS, Beal MF. Metabolomic profiling to develop blood biomarkers for Parkinson's disease. Brain 2008;131:389-396

Cleren C, Yang L, Lorenzo B, Calingasan NY, Schomer A, Sireci A, Wille EJ, Beal MF. Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. J Neurochem 2008; 104:1613-1621

Yang L, Calingasan NY, Lorenzo BJ, Beal MF. Attenuation of MPTP neurotoxicity by rolipram, a specific inhibitor of phosphodiesterase IV. Exp Neurol 2008;211:311-314

Yang L, Zhao K, Calingasan NY, Luo G, Szeto HH, Beal F. Mitochondria targeted peptides protect against 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine neurotoxicity. Antioxid Redox Signal 2009, Feb 9 (Epub ahead of print)